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L8: Entry 2 of 25

File: USPT

Oct 14, 2003

DOCUMENT-IDENTIFIER: US 6632430 B2

TITLE: Modulators of methylation for control of bacterial virulence

Brief Summary Text (4):

The importance of transmethylation reactions in metabolism in general has gained considerable recognition. PCT application WO96/20010 and U.S. Pat. No. 5,872,104, incorporated herein by reference, describe the use of methylation inhibitors to reduce the resistance of microorganisms to antibiotics. Heithoff, D. M., et al., Science (1999) 284:967-970 report the results of a study showing that *Salmonella typhimurium* which lacks DNA adenine methylase (Dam) were essentially avirulent and therefore could be used as live vaccines against murine typhoid fever. The authors concluded that Dam regulated the expression of at least 20 genes known to be induced during infection and noted that inhibitors of Dam were likely to be antimicrobials. It was earlier shown by Braaten, B. A., et al., Cell (1994) 76:577-588 that the methylation patterns associated with pyelonephritis-associated pili (Pap) DNA controlled gene expression in *E. coli*. Thus, it is clear that in bacteria, methylation status is significant in controlling metabolism, and thus infectivity in general. The importance of S-adenosyl-L-methionine (SAM) dependent transmethylation in viral infection has also been studied by Liu, S., et al., Antiviral Research (1992) 19:247-265.

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Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.63  
(O(6)-Methylguanine-DNA Methyltransferase); EC 2.5.1.18 (Glutathione Transferase)

Record Date Created: 19970407

Record Date Completed: 19970407

8/9/11

DIALOG(R) File 155: MEDLINE(R)

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13382816 PMID: 9055987

**Induction of ermSV by 16-membered-ring macrolide antibiotics.**

Kamimiya S; Weisblum B

Pharmacology Department, University of Wisconsin Medical School, Madison 53706, USA.

Antimicrobial agents and chemotherapy (UNITED STATES) Mar 1997, 41 (3) p530-4, ISSN 0066-4804 Journal Code: 0315061

Contract/Grant No.: AI-18283; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The erm family of 23S rRNA **adenine -N6- methyltransferases** confers resistance to all macrolide-lincosamide-streptograminB (MLS) antibiotics, but not all MLS antibiotics induce synthesis of Erm methyltransferase with equal efficiency in a given organism. The induction efficiency of a test panel of MLS antibiotics was studied by using two translational **attenuator** -lac reporter gene fusion constructs, one based on ermSV from Streptomyces viridochromogenes NRRL 2860 and the other based on ermC from Staphylococcus aureus RN2442. Four types of responses which were correlated with the macrolide ring size were seen, as follows: group 1, both ermSV and ermC were induced by the 14-membered-ring macrolides erythromycin, lankamycin, and matromycin, as well as by the lincosamide celesticetin; group 2, neither ermSV nor ermC was induced by the 12-membered-ring macrolide methymycin or by the lincosamide lincomycin or the streptogramin type B antibiotic ostreogrycin B; group 3, ermSV was selectively induced over ermC by the 16-membered-ring macrolides carbomycin, chalcomycin, cirramycin, kitasamycin, maridomycin, and tylasin; and group 4, ermC was selectively induced over ermSV by the 14-membered-ring macrolide megalomicin. These data suggest that the leader peptide determines the specificity of induction by different classes of MLS antibiotics and that for a given **attenuator**, a major factor which determines whether a given macrolide induces resistance is its size.

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: \*Anti-Bacterial Agents--pharmacology--PD; \*Genes, Bacterial--genetics--GE; \*Methyltransferases--genetics--GE; \*Streptomyces--genetics--GE; Anti-Bacterial Agents--chemistry--CH; Genes, Bacterial--physiology--PH; Genes, Reporter--genetics--GE; Genes, Reporter--physiology--PH; Lac Operon--genetics--GE; Macrolides; Microbial Sensitivity Tests; **Plasmids**--genetics--GE; Streptomyces--drug effects--DE; Streptomyces--enzymology--EN

CAS Registry No.: 0 (Anti-Bacterial Agents); 0 (Macrolides); 0 (Plasmids)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.66 (rRNA (adenosine -O-2')- methyltransferase )

Record Date Created: 19970602

Record Date Completed: 19970602

8/9/12

DIALOG(R) File 155: MEDLINE(R)

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13252537 PMID: 8918477

**Chromosomal replication incompatibility in Dam methyltransferase**

**deficient Escherichia coli cells.**

Lobner-Olesen A; von Freiesleben U

University of Colorado at Boulder, Dept of Molecular Cellular and  
Developmental Biology 80309, USA.

EMBO journal (ENGLAND) Nov 1 1996, 15 (21) p5999-6008, ISSN  
0261-4189 Journal Code: 8208664

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Dam methyltransferase deficient Escherichia coli cells containing minichromosomes were constructed. Free **plasmid** DNA could not be detected in these cells and the minichromosomes were found to be integrated in multiple copies in the origin of replication (oriC) region of the host chromosome. The absence of the initiation cascade in Dam- cells is proposed to account for this observation of apparent incompatibility between **plasmid** and chromosomal copies of oriC. Studies using oriC-pBR322 chimeric **plasmids** and their deletion derivatives indicated that the seqA2 **mutation** was found to overcome the incompatibility phenotype by increasing the cellular oriC copy number 3-fold thereby allowing minichromosomes to coexist with the chromosome. The replication pattern of a wild-type strain with multiple integrated minichromosomes in the oriC region of the chromosome, led to the conclusion that initiation of DNA replication commences at a fixed cell mass, irrespective of the number of origins contained on the chromosome.

Descriptors: Chromosomes, Bacterial--metabolism--ME; \*DNA Replication; \*Escherichia coli--genetics--GE; \*Escherichia coli--metabolism--ME; \*Site-Specific DNA- **Methyltransferase** ( Adenine -Specific)--metabolism--ME ; Chromosomes, Bacterial--genetics--GE; DNA Methylation; DNA, Bacterial --biosynthesis--BI; DNA, Bacterial--genetics--GE; Genes, Bacterial; **Mutation** ; **Plasmids** --genetics--GE; **Plasmids** --metabolism--ME; Replication Origin; Site-Specific DNA- **Methyltransferase** ( Adenine -Specific)--genetics--GE; Transformation , Genetic

CAS Registry No.: 0 (DNA, Bacterial); 0 (Plasmids)

Enzyme No.: EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72  
(Site-Specific DNA- **Methyltransferase** ( Adenine -Specific))

Record Date Created: 19970108

Record Date Completed: 19970108

product of a previously undescribed gene, which we named *mrr* (methylated adenine recognition and restriction). We suggest that *mrr* encodes an endonuclease that cleaves DNA containing N6-methyladenine and that DNA double-strand breaks induce the SOS response. Cytosine methylases **foreign** to *E. coli* (*MspI* [*meCCGG*], *HaeIII* [*GGmeCC*], *BamHI* [*GGATmeCC*], *HhaI* [*GmeCGC*], *BsuRI* [*GGmeCC*], and *M.Spr*) also induced SOS, whereas one indigenous to *E. coli* (*EcoRII* [*CmeCA/TGG*]) did not. SOS induction by cytosine methylation required the *rglB* locus, which encodes an endonuclease that cleaves DNA containing 5-hydroxymethyl- or 5-methylcytosine (E. A. Raleigh and G. Wilson, Proc. Natl. Acad. Sci. USA 83:9070-9074, 1986).

Tags: Support, U.S. Gov't, Non-P.H.S.; Support, U.S. Gov't, P.H.S.

Descriptors: DNA Damage; \*DNA Repair; \*Escherichia coli--genetics--GE; \*Methyltransferases--metabolism--ME; \*SOS Response (Genetics); \*Site-Specific DNA- **Methyltransferase** ( **Adenine** -Specific); 5-Methylcytosine; Adenine-analogs and derivatives--AA; Adenine--physiology--PH; Bacteriophage lambda--genetics--GE; Cloning, Molecular; Cytosine--analogs and derivatives--AA; Cytosine--physiology--PH; DNA (Cytosine-5)-Methyltransferase--metabolism--ME; DNA Transposable Elements; Lysogeny; Methyltransferases--genetics--GE; **Mutation** ; Promoter Regions (Genetics)

CAS Registry No.: 0 (DNA Transposable Elements); 443-72-1 (6-methyladenine); 554-01-8 (5-Methylcytosine); 71-30-7 (Cytosine); 73-24-5 (Adenine)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.- (DNA modification methylase *HinfI*); EC 2.1.1.37 (DNA (Cytosine-5)-Methyltransferase); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** ( **Adenine** -Specific))

Record Date Created: 19870807

Record Date Completed: 19870807

8/9/83

DIALOG(R) File 155: MEDLINE(R)

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07288542 PMID: 3536887

**Biological role of DNA methylation: sequence-specific single-strand breaks associated with hypomethylation of GATC sites in Escherichia coli DNA.**

Szyf M; Meisels E; Razin A

Journal of bacteriology (UNITED STATES) Dec 1986, 168 (3) p1487-90, ISSN 0021-9193 Journal Code: 2985120R

Contract/Grant No.: GM 20483; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The effect of methylation of GATC sites in *Escherichia coli* DNA on the formation of single-strand breaks was studied with *dam*+, *dam* **mutant**, and *Dam*-overproducer strains. Single-strand breaks have been observed in *dam* **mutant** cells predominantly at TpT and, to a lesser extent, at CpC. In *dam* **mutant** cells harboring pTP166 (a **plasmid** containing the *dam* gene), no such nicks were observed.

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: \*DNA Damage; \*DNA, Bacterial--metabolism--ME; \*DNA, Single-Stranded--metabolism--ME; \*Escherichia coli--metabolism--ME; \*Methyltransferases--physiology--PH; Base Sequence; Methylation; **Plasmids**; Site-Specific DNA- **Methyltransferase** ( **Adenine** -Specific)

CAS Registry No.: 0 (DNA, Bacterial); 0 (DNA, Single-Stranded); 0 (Plasmids)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** ( **Adenine** -Specific))

Record Date Created: 19870114

Record Date Completed: 19870114



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<b>Designations:</b>	NADC-D60aroA-	<b>Isolation:</b>	lung from cow with pneumonic pasteurellosis [38863]			
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<b>Applications:</b>	used to produce live, attenuated veterinary vaccines against <b><i>Pasteurella haemolytica</i></b> [38863]					
<b>References:</b>	38863: Construction of <b><i>Pasteurella haemolytica</i></b> vaccines. US Patent 5,733,780 dated Mar 31 1998 61435: Briggs RE, Tatum FM . Construction of <b><i>Pasteurella haemolytica</i></b> vaccines. US Patent 5,733,780 dated Mar 31 1998					

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<input type="checkbox"/>	L1	(heterologous or heter-ologous or foreign or nonnative or non-native).clm.	10571
<input type="checkbox"/>	L2	(heterologous or heter-ologous or foreign or nonnative or non-native or exogenous).clm.	11879
<input type="checkbox"/>	L3	L2 and dam and adenine	41
<input type="checkbox"/>	L4	L2 and adenine	956
<input type="checkbox"/>	L5	L4 and (methylase or methyltransferase or methyl-transferase or dam or (dna near adenine))	133

END OF SEARCH HISTORY

0197305 DBR Accession No.: 96-07446 PATENT  
**Attenuated virus containing additional methylation sites in its genome -**  
**HIV virus-1, SIV virus or HTLV-I virus attenuation by deletion mutation**  
**for use as recombinant vaccine; DNA probe and DNA primer**  
AUTHOR: Nyce J W  
CORPORATE SOURCE: Greenville, NC, USA.  
PATENT ASSIGNEE: Univ.East-Carolina 1996  
PATENT NUMBER: WO 9611280 PATENT DATE: 960418 WPI ACCESSION NO.:  
96-209861 (9621)  
PRIORITY APPLIC. NO.: US 319974 APPLIC. DATE: 941007  
NATIONAL APPLIC. NO.: WO 95US13219 APPLIC. DATE: 951005  
LANGUAGE: English

...ABSTRACT: an attenuating deletion mutation. Also new are: DNA (I) encoding the attenuated virus; an expression **vector** (baculo virus) containing (I); a **host** cell containing (I) and expressing attenuated virus, where the **host** cell does not **methylate** the **DNA** sufficiently to inactivate expression of the encoded virus genome due to treatment of the cell...

... inhibitor (5-azadeoxyctidine or 5-azacytidine); a pharmaceutical formulation of the attenuated virus; a recombinant **vaccine** and vaccination method; a method for making the attenuated virus by transforming a mammal or insect cell with a **vector**; and a DNA probe or DNA primer for detecting attenuated virus. (41pp)

DESCRIPTORS: HIV virus-1, SIV virus, HTLV-I virus attenuation, deletion mutant, multiple **methylation** site, **DNA** probe, **DNA** primer, baculo virus **vector** expression in mammal, insect cell culture, pot. recombinant **vaccine** mutation retro virus leuko virus lenti virus T-lymphocyte leukemia virus foamy virus AIDS animal...

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L5: Entry 39 of 133

File: USPT

Jun 18, 2002

US-PAT-NO: 6406885

DOCUMENT-IDENTIFIER: US 6406885 B1

\*\* See image for Certificate of Correction \*\*

TITLE: Plants and plant cells expressing histidine tagged intimin

DATE-ISSUED: June 18, 2002

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stewart, Jr.; C. Neal	Greensboro	NC		
McKee; Marian L.	Great Falls	VA		
O'Brien; Alison D.	Bethesda	MD		
Wachtel; Marian R.	Gaithersburg	MD		

## ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE	CODE
Henry M. Jackson Foundation for the Advancement of Military Medicine	Rockville	MD			02	

APPL-NO: 09/ 696188 [PALM]

DATE FILED: October 26, 2000

## PARENT-CASE:

## CROSS-REFERENCE TO RELATED APPLICATION

This is a division of application Ser. No. 08/840,466, filed Apr. 18, 1997 now U.S. Pat. No. 6,261,561, which is a continuation of provisional application Ser. No. 60/015,657, filed Apr. 19, 1996 and provisional application Ser. No. 60/015,938, filed Apr. 22, 1996, all of which are specifically incorporated herein by reference.

This application is related to provisional applications entitled HISTIDINE-TAGGED INTIMIN AND METHODS OF USING INTIMIN TO STIMULATE AN IMMUNE RESPONSE AND AS AN ANTIGEN CARRIER WITH TARGETING CAPABILITY, of inventors Marian McKee, Alison O'Brien, and Marian Wachtel Provisional Application No. 60/015,937; filed on Apr. 19, 1996, and Provisional Application No. 60/015,938, filed on Apr. 22, 1996; said applications are incorporated herein by reference.

INT-CL: [07] C12 P 21/06

US-CL-ISSUED: 435/69.1; 435/69.3, 435/410, 435/420, 536/23.1, 536/23.2, 536/23.7

US-CL-CURRENT: 435/69.1; 435/410, 435/420, 435/69.3, 536/23.1, 536/23.2, 536/23.7

FIELD-OF-SEARCH: 435/252.2, 435/252.8, 435/320.1, 435/69.3, 435/69.1, 435/91.41, 435/410, 435/420, 536/23.7, 536/23.1, 536/23.2, 800/298, 800/300, 800/301, 800/302, 800/317.2, 800/322

PRIOR-ART-DISCLOSED:

## U.S. PATENT DOCUMENTS

Search Selected	Search ALL	Clear
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PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
<input type="checkbox"/> <u>4736017</u>	April 1988	O'Hanley et al.	
<input type="checkbox"/> <u>4740585</u>	April 1988	Schmidt et al.	
<input type="checkbox"/> <u>5049500</u>	September 1991	Arnizen et al.	
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<input type="checkbox"/> <u>5532142</u>	July 1996	Johnston et al.	
<input type="checkbox"/> <u>5747293</u>	May 1998	Dougan et al.	
<input type="checkbox"/> <u>5759551</u>	June 1998	Ladd et al.	
<input type="checkbox"/> <u>5798260</u>	August 1998	Tarr et al.	

## FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
2078716	March 1994	CA	
0 222 835	May 1987	EP	
0282042	September 1988	EP	
90/02484	March 1990	WO	
92/02820	February 1992	WO	
95/15678	June 1994	WO	
94/20135	September 1994	WO	
94/25060	November 1994	WO	
96/00233	January 1996	WO	
96/12801	May 1996	WO	

## OTHER PUBLICATIONS

Beebakhee et al., "Cloning and Nucleotide Sequence of the eae Gene Homologue from Enterohemorrhagic *Escherichia coli* Serotype 0157:H7," *FEMS Microbiol. Ltrs.*, 91:63-68 (1992).

Dalsgaard et al., "Plant-Derived Vaccine Protects Target Animals Against a Viral Disease," *Nature Biotechnology*, 15:248-252 (1997)..

Abstract: C. Neal Stewart, Jr.; Marian R. Wachtel; Stephen A. Mabon; William B. Warrick; and Alison D. O'Brien, "Expression of Enterohemorrhagic *Escherichia coli* Intimin in Transgenic Plants: An Edible Anti-EHEC 0157:H7 Vaccine Candidate," VTEC Meeting, Baltimore, Maryland, Jun. 22-26, 1997.

Abstract: Marian R. Wachtel; Lisa J. Gansheroff; and Alison D. O'Brien, "Structure-Function Analysis, Purification, and Immunoreactivity of Enterohemorrhagic *Escherichia coli* Intimin," VTEC Meeting, Baltimore, Maryland, Jun. 22-26, 1997.

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### Excipient

- (n.) An exceptor.
- (n.) An inert or slightly active substance used in preparing remedies as a vehicle or medium of administration for the medicinal agents.
- (v. t.) Taking an exception.

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## excipient

<[chemistry](#), [pharmacology](#)> Any more or less inert substance added to a prescription in order to confer a suitable consistency or form to the drug, a vehicle.

Origin: L. *Excipiens*, *capere* = to take

(18 Nov 1997)

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### excipient

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**ex·cip·i·ent** [ ik síppee nt ]  
(plural **ex·cip·i·ents**)

**noun**

**something combined with a drug:** an inert substance, for example, starch or gum arabic, that is combined with a drug to make it easier to administer

[Early 18th century. From Latin *excipient-*, the present participle stem of *excipere* “to receive, take out” (see *except*).]

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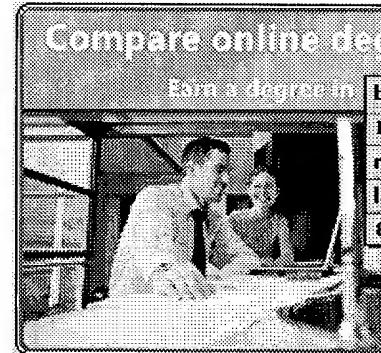
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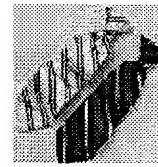
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12684915 PMID: 7607497

**Expression of the SalI restriction-modification system of Streptomyces albus G in Escherichia coli.**

Alvarez M A; Gomez A; Gomez P; Rodicio M R

Departamento de Biología Funcional, Universidad de Oviedo, Spain.

Gene (NETHERLANDS) May 19 1995, 157 (1-2) p231-2, ISSN 0378-1119

Journal Code: 7706761

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The salIR and salIM genes of *Streptomyces albus G* encode the restriction endonuclease (ENase) and DNA methyltransferase (MTase) of the SalI restriction-modification (R-M) system. In *S. albus G*, the genes constitute an operon that is mainly transcribed from a promoter located upstream from salIR, the first gene of the operon. In addition, a second promoter, at the 3' end of salIR, allows independent transcription of the MTase gene. Expression of salIR and salIM in *Escherichia coli* was investigated. The ENase gene was not expressed in the **heterologous** host, probably due to inactivity of the main promoter of the salI operon. In contrast to salIR, salIM was functional in *E. coli*. Preliminary S1 nuclease mapping experiments suggest that the **alternative** promoter of the MTase gene can initiate transcription in the **heterologous**, as well as in the homologous host.

Tags: Support, Non-U.S. Gov't

Descriptors: Deoxyribonucleases, Type II Site-Specific--biosynthesis--BI; \*Recombinant Proteins--biosynthesis--BI; \*Site-Specific DNA- **Methyltransferase** ( **Adenine** -Specific)--biosynthesis--BI; \**Streptomyces*--enzymology--EN ; Cloning, Molecular--methods--MT; Deoxyribonucleases, Type II Site-Specific--genetics--GE; *Escherichia coli*--metabolism--ME; Gene Expression; Genes, Bacterial; Operon; Promoter Regions (Genetics); Site-Specific DNA- **Methyltransferase** ( **Adenine** -Specific)--genetics--GE; *Streptomyces*--genetics--GE

CAS Registry No.: 0 (Recombinant Proteins)

2/2  
J. C. M.

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DATE: Tuesday, November 02, 2004

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L15: Entry 36 of 40

File: DWPI

Jul 4, 2002

DERWENT-ACC-NO: 2002-635659

DERWENT-WEEK: 200424

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TITLE: Attenuated strain of bacteria useful for eliciting immune response in an individual, has altered DNA adenine methylase activity

INVENTOR: HEITHOFF, D M; LOW, D A ; MAHAN, M J ; SINSHEIMER, R L

PATENT-ASSIGNEE: HEITHOFF D M (HEITI), LOW D A (LOWDI), MAHAN M J (MAHAI), SINSHEIMER R L (SINSI)

PRIORITY-DATA: 2001US-0927896 (August 9, 2001), 1999US-183043P (February 2, 1999), 1999US-198250P (May 5, 1999), 2000US-0495614 (February 1, 2000), 2000US-0612116 (July 7, 2000)

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## PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
<input type="checkbox"/> <a href="#">US 20020086032 A1</a>	July 4, 2002		044	A61K039/02

## APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
US20020086032A1	February 2, 1999	1999US-183043P	Provisional
US20020086032A1	May 5, 1999	1999US-198250P	Provisional
US20020086032A1	February 1, 2000	2000US-0495614	CIP of
US20020086032A1	July 7, 2000	2000US-0612116	CIP of
US20020086032A1	August 9, 2001	2001US-0927896	

INT-CL (IPC): [A61 K 39/02](#); [C12 N 1/21](#)

RELATED-ACC-NO: 2000-532863;2002-557291 ;2002-598710 ;2002-635674 ;2002-690113

ABSTRACTED-PUB-NO: US20020086032A

## BASIC-ABSTRACT:

NOVELTY - An attenuated strain (I) of bacteria, comprising altered DNA adenine methylase (Dam) activity, such that the bacteria are attenuated, is new.

## DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) administering (M1) to a subject capable of generating an immune response, a composition comprising a pharmaceutically acceptable excipient and an immunogenic

dose of attenuated bacteria comprising altered Dam activity relative to a Wild-type bacteria, and allowing the composition to remain in the subject for a time and under conditions to allow the subject to generate an immune response to the attenuated bacteria and produce antibodies specific to the attenuated bacteria; and

(2) an immunogenic composition (II), comprising a pharmaceutically acceptable excipient, and live bacteria comprising altered Dam activity, where the altered activity reduces virulence relative to the bacteria with wild-type Dam activity.

ACTIVITY - None given.

MECHANISM OF ACTION - Vaccine. Strains which demonstrated attenuation as a result of intraperitoneal or oral challenge of BALB/c mice were further tested for protective immunity against subsequent challenge by the wild-type strain at 105 I.P. or 109 orally. BALB/c mice were perorally immunized through gastrointubation with a dose of 10+9 Dam- S.typhimurium. Five weeks later, the immunized mice were challenged perorally with 10+9 wild-type S.typhimurium. After five weeks, surviving mice were challenged with the wild-type 14028 strain. Survival for four weeks post challenge was deemed full protection. The data demonstrated the potential use of (I) in developing vaccine strains. Since Dam- mutants were highly attenuated, it was determined whether Dam- Salmonella could serve as a live attenuated vaccine. The result showed that all (17/17) mice immunized with a S.typhimurium Dam- insertion strain survived a wild-type challenge of 10+4 above the LD50, whereas all nonimmunized mice (12/12) died following challenge.

USE - M1 is useful for eliciting an immune response and producing antibodies specific to (I). (I) or (II) is useful for eliciting an immune response in an individual, by administering (I) or (II) to an individual, and allowing (I) or (II) to remain in the individual for a time and under conditions to allow the individual to generate an immune response (claimed). (I) is useful for producing antibodies, producing higher concentration of B-cell, and for vaccinating a host against a pathogenic microorganism or a spectrum of pathogenic microorganisms.

ABSTRACTED-PUB-NO: US20020086032A

EQUIVALENT-ABSTRACTS:

CHOSEN-DRAWING: Dwg.0/9

DERWENT-CLASS: B04 C06 D16

CPI-CODES: B04-F10; B04-G01; B04-L0400E; B11-C07A; B11-C08E1; B12-K04E; B14-S11; C04-F10; C04-G01; C04-L0400E; C11-C07A; C11-C08E1; C12-K04E; C14-S11; D05-A02; D05-H04; D05-H07;

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L1: Entry 2 of 46

File: USPT

Jun 1, 2004

DOCUMENT-IDENTIFIER: US 6743609 B1

TITLE: Linoleate isomerase

Detailed Description Text (147):

The PCR product of 1.9 kb was cloned as blunt ends at the SrfI site into pPCR-Script Amp SK and transformed into cells of *E. coli* strain NovaBlue. Since dam methylation in this host prevents BclI digestion, the recombinant plasmid was transformed into cells of *E. coli* strain GM2163, which is a dam minus strain. Recombinant plasmid DNA was digested with the restriction enzymes NdeI and BclI and ligated to the vector PBHAI which had been digested with NdeI and BamHI. Recombinant plasmid DNA was digested with SacI to remove the *E. coli* portion of the vector, recircularized, and transformed into *B. subtilis* 23856.

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L1: Entry 4 of 46

File: USPT

Mar 16, 2004

DOCUMENT-IDENTIFIER: US 6706860 B2

TITLE: Toxins

Detailed Description Text (26):

For truncation of the *isp2* gene, a fragment containing the gene was cut out of pSLIB120/ISP using *Bst*BI and *Pml*1. Following the GPS reaction, this fragment was then ligated into the pSLIB120/ISP vector, cut with the same enzymes. Recombinant *E. coli* colonies were PCR-screened in order to estimate the position of the transposon insertion site. A set of 10 clones with the transposon at different positions in the second half (3' half) of the *isp2* gene was selected. Plasmid DNA of these clones was transformed in a *dcm* and *dam* methylase negative *E. coli* strain GM2163. Plasmid DNA isolated from this strain was then transformed in a crystal-minus Bt strain. Supernatant was prepared and bioassayed, using supernatant from the Bt strain transformed with pSLIB120/ISP as a positive control and supernatant from the crystal-minus Bt strain as a negative control. For truncation of the *isp1* gene, a fragment was cut out of pSLIB120/ISP using *Pml*1 and *Eco*RI. The same methodology was followed as for the truncation of the *isp2* gene.

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L1: Entry 5 of 46

File: USPT

Mar 16, 2004

DOCUMENT-IDENTIFIER: US 6706501 B1

**TITLE: Polynucleotide encoding a *propionibacterium* linoleate isomerase and uses thereof**

Detailed Description Text (186):

The PCR product of 1.9 kb was cloned as blunt ends at the SrfI site into pPCR-Script Amp SK and transformed into cells of *E. coli* strain NovaBlue. Since dam methylation in this host prevents BclI digestion, the recombinant plasmid was transformed into cells of *E. coli* strain GM2163, which is a dam minus strain. Recombinant plasmid DNA was digested with the restriction enzymes NdeI and BclI and ligated to the vector pBHAI which had been digested with NdeI and BamHI. Recombinant plasmid DNA was digested with SacI to remove the *E. coli* portion of the vector, recircularized, and transformed into *B. subtilis* 23856.

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L19: Entry 7 of 7

File: USPT

Dec 28, 1993

DOCUMENT-IDENTIFIER: US 5273744 A

TITLE: Vaccines for the protection of animals against theileria infection

Detailed Description Text (106):

The gene contains a BclI site 23 nucleotides in from the N-terminal end (FIG. 1). Since BclI digestion is blocked by the dam methylase, plasmid 2 is grown in a methylase-deficient strain of E.coli such as NK5772. Plasmid 2 is prepared from NK5772 and is digested with BclI and a synthetic adaptor is attached to the DNA.

CLAIMS:

2. A vaccine for inducing immunoprotection in bovine animal species against infections of Theileria parva comprising pharmaceutically acceptable excipients and a substantially pure and isolated antigen having the amino acid sequence set forth in FIG. 1 said antigen present in an amount effective to induce immunoprotection against infection by Theileria parva when administered to the animal.
3. A vaccine of claim 2 wherein the antigen is glycosylated.
4. A vaccine of claim 2 wherein the antigen is not glycosylated.
5. A vaccine of claim 2 wherein the animals are cattle.
6. A vaccine of claim 2 wherein the antigen is a recombinant protein.
7. A vaccine of claim 2 wherein the substantially pure and isolated antigen is at least 75% pure.
8. A method for protecting bovine animal species from infection by Theileria parva comprising the administration of a vaccine comprising an amount of a substantially pure and isolated antigen having the amino acid sequence set forth in FIG. 1 said amount effective for inducing immunoprotection against infection by Theileria parva in the animal.

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GM 3  
GM 10  
PU 10

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L9: Entry 41 of 41

File: USPT

*Atte p. David*  
Jan 17, 1989

DOCUMENT-IDENTIFIER: US 4798791 A

TITLE: Vector for high level gene expression

Detailed Description Text (15):

Plasmid pGX2257 DNA was prepared from *E. coli* strain GX3003 (pGX2257) that contains a DNA adenine methylase (dam) mutation. *E. coli* strain GX1731 (pGX2257) has been deposited at the U.S. Department of Agriculture, Northern Regional Research Laboratory, Peoria, Ill., with accession No. NRRL B-15771. Any *E. coli* host with a dam mutation (A. Bale, M. D'Alarcao and M. G. Marinus, Mutation Research, 59:157165 (1979)) that is a lambda phage lysogen can be utilized in place of GX3003 for this method. The pGX2257 DNA (15 .mu.g) prepared in a dam host was digested with 8 units of HpaI endonuclease in 100 .mu.l of the buffer recommended by the manufacturer at 37.degree. C. for two hours. The DNA solution was then extracted with a 1:1 mixture of water saturated phenol and cloroform (adjusted to pH 8.0 with tris-[hydroxymethyl]-aminomethane base). The aqueous phase was adjusted to a 0.2 M sodium acetate pH 5.5 and 2.5 volume of 95% ethanol was added to precipitate the DNA. The ethanol solution was frozen using dry ice then centrifuged at 15,000 X G to precipitate the DNA. The DNA was dissolved in 30 .mu.l H<sub>2</sub>O to give a DNA concentration of 0.5 .mu.g/.mu. l.

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*CD167*

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L15: Entry 20 of 40

File: USPT

Jul 1, 2003

US-PAT-NO: 6585975

DOCUMENT-IDENTIFIER: US 6585975 B1

TITLE: Use of *Salmonella* vectors for vaccination against *helicobacter* infection

DATE-ISSUED: July 1, 2003

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kleanthous; Harold	Westford	MA		
Londono-Arcila; Patricia	London			GB
Freeman; Donna	Cambridge			GB
Lee; Cynthia K.	Needham	MA		
Monath; Thomas P.	Harvard	MA		

## ASSIGNEE-INFORMATION:

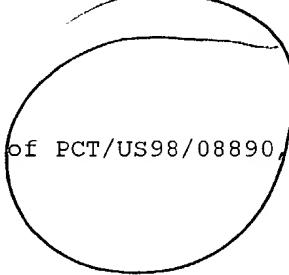
NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Acambis, Inc.	Cambridge	MA			02

APPL-NO: 09/ 431705 [PALM]

DATE FILED: November 1, 1999

## PARENT-CASE:

This application is a continuation-in-part of PCT/US98/08890, which s filed on Apr. 30, 1998.


INT-CL: [07] A61 K 39/02

US-CL-ISSUED: 424/200.1; 424/234.1, 435/69.1, 435/6, 514/44, 536/23.5

US-CL-CURRENT: 424/200.1; 424/234.1, 435/6, 435/69.1, 514/44, 536/23.5

FIELD-OF-SEARCH: 424/234.1, 424/200.1, 536/23.5, 514/44, 435/184, 435/6, 435/69.1

## PRIOR-ART-DISCLOSED:

## U.S. PATENT DOCUMENTS

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PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
<input type="checkbox"/> <u>4888170</u>	December 1989	Curtiss, III	
<input type="checkbox"/> <u>5538729</u>	July 1996	Czinn et al.	424/234.1

<input type="checkbox"/> <u>5547664</u>	August 1996	Charles	424/93.2
<input type="checkbox"/> <u>5683700</u>	November 1997	Charles et al.	
<input type="checkbox"/> <u>5783196</u>	July 1998	Noriega et al.	424/234.1
<input type="checkbox"/> <u>5843426</u>	December 1998	Miller et al.	424/93.2
<input type="checkbox"/> <u>5843460</u>	December 1998	Labigne et al.	424/234.1
<input type="checkbox"/> <u>5877159</u>	March 1999	Powell et al.	514/44
<input type="checkbox"/> <u>5888799</u>	March 1999	Curtiss, III	
<input type="checkbox"/> <u>5928865</u>	July 1999	Covacci	435/6
<input type="checkbox"/> <u>5985631</u>	November 1999	Soman et al.	435/184
<input type="checkbox"/> <u>6005090</u>	December 1999	Dodge et al.	536/23.5
<input type="checkbox"/> <u>6024961</u>	February 2000	Curtiss, III et al.	
<input type="checkbox"/> <u>6030624</u>	February 2000	Russell	424/200.1
<input type="checkbox"/> <u>6126938</u>	October 2000	Guy et al.	424/184.1
<input type="checkbox"/> <u>6383496</u>	May 2002	Curtiss, III et al.	424/200.1

## FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
835928	April 1998	EP	
9215688	September 1992	WO	
9318150	September 1993	WO	
9522987	August 1995	WO	
9640893	December 1996	WO	
9702835	January 1997	WO	
WO 99/21959	May 1999	WO	

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 Lugtenburg, T. Safety and efficacy of oral or combined oral and parenteral immunization with inactivated or live avirulent bacteria against *Salmonella typhimurium* in the calf., *Ludwig-Maximilians Universitat, Munchen* (abstrct of thesis), 1981.\*

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Krachenbuhl, JP, Infectious Disease Weekly, pN/A, Jul. 8, 1996, Vaccine Development "Vaccines of Tomorrow: The Hope of Science."

ART-UNIT: 1645

PRIMARY-EXAMINER: Smith; Lynette R. F.

ASSISTANT-EXAMINER: Portner; Ginny Allen

ATTY-AGENT-FIRM: Clark & Elbing LLP

ABSTRACT:

The invention provides a method of immunization against Helicobacter, involving mucosal administration of an attenuated Salmonella vector including a nucleic acid molecule encoding a Helicobacter antigen, and parenteral administration of a soluble soluble Helicobacter antigen, co-administered with a suitable parenteral adjuvant. Also provided by the invention are attenuated Salmonella vectors for use in this method.

15 Claims, 10 Drawing figures

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